REMARKS

Claims 1-19 are pending. Claims 7-19 have been withdrawn from consideration. Applicants have amended claims 1 and 7. Support for these amendments can be found in the specification, for example, at page 4, lines 1-6 and lines 25-30. No new matter has been added by these amendments.

The Invention

Pro-urokinase (pro-UK) is a natural zymogen that activates plasminogen, to form plasmin, which in turn activates pro-UK to UK. The present application discloses methods of treatment (e.g., treatment of stroke) using a mutant form of pro-UK, the "M5" mutant. Once activated, the M5 mutant has a catalytic efficiency that is higher than UK. In a plasma milieu *in vitro* or *in vivo,* it can induce clot lysis that is twice as rapid as pro-UK (or tissue plasminogen activator (t-PA)). The M5 mutant is more efficient than pro-UK or t-PA, e.g., the total amount of M5 needed is less than what is needed when using pro-UK or t-PA.

Of particular significance, the M5 mutant is selectively targeted to plasminogen bound to occlusive "bad" clots ("occlusive clots"), such as occlusive thrombi or emboli, that include partially degraded fibrin, while being relatively inactive against plasminogen bound to "good" clots ("hemostatic clots") that comprise intact fibrin (see, e.g., application at pages 3-4). The good hemostatic clots seal injured blood vessels, such as those present in a wound, at an incision after surgery, or in a healing blood vessel that has leaked or ruptured in the brain potentially causing a stroke. In contrast to the M5 mutant, pro-UK at therapeutic doses becomes unstable and is readily converted to UK, which activates plasminogen indiscriminately, that is, it will cleave plasminogen which is unbound or bound to good clots and plasminogen bound to bad clots. When this occurs, hemophilia-like side effects occur, as well as bleeding due to the degradation of good clots. T-PA, another clot lysis agent, can also cause hemorrhagic side effects in patients which have been specifically related to the degradation of good clots and its use has therefore been limited.

As a result of its specificity for bad clots and its sparing of good clots (hemostatic fibrin), therapeutic use of the M5 mutant provides significant advantages over the use of pro-UK or t-PA. For example, use of the M5 mutant minimizes or avoids the side effect of cleaving good clots that is caused by pro-UK (due to its ready conversion to UK) or t-PA. Based on these newly discovered characteristics, the M5 mutant can be administered immediately to a patient exhibiting symptoms of stroke without first determining the cause of the stroke, because the M5 mutant has no negative side effects.

In contrast, diagnostic procedures that can dramatically delay the start of treatment, such as CT scanning, must be performed prior to administering pro-UK or t-PA, because if a patient's stroke is due to vessel rupture and hemorrhage instead of vessel occlusion, administration of pro-UK or t-PA will cause additional bleeding and can potentially kill the patient. Due to M5's preference for "bad" clots, such diagnostic procedures are not needed prior to administration of the M5 mutant because the good clots are not vulnerable to M5 cleavage. As a result, no delay occurs prior to initiating stroke treatment with the M5 mutant.

Restriction Requirement

Applicants maintain their traversal of the restriction requirement, because the Office Action indicates a seeming misunderstanding of the invention. The Examiner is, of course, correct that the three disorders covered by the three Groups are distinct. However, applicants submit that the claims in Groups I, II, and III can easily be examined together, because all three methods have the same mode of operation, i.e., the M5 mutant form of pro-UK lyses blood clots that cause the symptoms of stroke or a heart attack and any occlusive blood clots that cause problems after surgery. The M5 selectively lyses these occlusive clots while not lysing hemostatic clots that close wounds.

The Office Action alleges that M5 must cross the blood-brain barrier to treat stroke, yet the M5 stays in the blood when administered intravenously. In heart attacks, administration is also intravenous, not oral. For surgery, administration is intravenous, because a damaging clot can travel anywhere in the bloodstream. Thus, the mode of operation of M5 is the same in each

of the three different methods, so all claims can be examined together without an undue burden on the Examiner.

35 U.S.C. § 103

Claims 1 and 3-6

The Office Action alleges that claims 1 and 3-6 are obvious in light of Liu et al. (U.S. Pat. No. 5,472,692), Brearley et al. (U.S. App. No. 2002/0098179), and Pinsky (U.S. App. No. 2002/0138858). Applicants respectfully disagree and submit that the Office has failed to establish a *prima facie* case of obviousness with respect to these claims, because these references, alone or in combination, fail to provide a motivation to combine the references.

As amended, claim 1 recites the step of "determining that the person potentially has had a stroke based on observing one or more symptoms of stroke to make an initial diagnosis without determining the cause of the stroke," indicating that treatment with M5 can be initiated upon presentation of stroke symptoms without the need to first confirm the cause.

Liu describes the creation of pro-UK mutants, including the M5 mutant, and *in vitro* characterization of several of the mutants. As the Office Action concedes, Liu fails to describe the use of the M5 mutant to treat stroke. Nor could it have, because when the present inventors (who are also the inventors of M5) filed the Liu et al. patent application, they did not know of the specific characteristics of M5 described in the present application. For example, at the time the Liu et al. patent was filed, it was not known that M5 is selective for bad occlusive clots, but spares good hemostatic clots, or that M5 can be administered immediately to a patient exhibiting symptoms of stroke without the need for prior diagnostic testing to determine whether the stroke is caused by a clot or a ruptured blood vessel.

Liu states, "[b]oth pro-UK and UK can cause non-specific plasminogen activation, which leads to the degradation of fibrin, fibrinogen (fibrinogenolysis), and certain parts of platelets and blood vessel walls, and hemorrhagic diathesis," (column 1, lines 15-19; emphasis added), and "[a]s a result, these pro-UK mutants cause lower non-specific plasminogen activation and bleeding complications than native pro-UK when administered to a patient" (column 1, lines 60-

63; emphasis added). The "bleeding complications" referred to in these quoted passages refer to **systemic bleeding** caused by non-specific plasminogen activation. As discussed above, when pro-UK cleaves (e.g., activates) plasminogen to form plasmin, the plasmin cleaves pro-UK to form UK, leading to non-specific plasminogen activation. Due to the non-specificity of its activity, UK cleaves plasminogen bound to any clots and also unbound in the circulation. As a result, systemic bleeding can occur. Liu predicted that its pro-UK mutants would cause less **systemic bleeding** because they retain their specificity for plasminogen bound to any clots and would not activate plasminogen present in the circulation:

> These pro-UK mutants are superior thrombolytic agents compared to native pro-UK, because they are promoted by fibrin to the same extent as native pro-UK, and therefore have the same fibrinolytic efficiency, but have a far greater specificity for fibrin-bound plasminogen than native pro-UK, because they are truly inert in plasma. (column 1, line 64 to column 2, line 2; emphasis added)

In contrast, the present application identifies a new, novel specificity of M5 that was not known at the time of the Liu patent. Namely, the M5 mutant can differentiate between plasminogen bound to good (hemostatic) clots versus plasminogen bound to bad (occlusive) clots. Further, the M5 mutant cleaves plasminogen bound to the bad clots while sparing the good clots. As a result of this novel property described in the present application, the M5 mutant can be administered immediately to a stroke patient. Absent the knowledge of M5's unique property, one of ordinary skill in this field would not have administered a pro-UK mutant as described in the Liu patent to a stroke victim without confirming an initial stroke diagnosis with a diagnostic test to determine the cause of the stroke. Bleeding due to the systemic induction of a hemorrhagic state (secondary to non-specific plasminogen activation) and that due to direct lysis of hemostatic fibrin (good fibrin) are not the same and cannot be equated.

Brearley describes the use of a combination therapy to treat stroke. The combination therapy includes the use of at least one Neutrophil Inhibitory Factor (NIF) and at least one other agent, which can be a thrombolytic agent. Brearley also identifies problems with the current therapies, which employ a single agent such as pro-UK and t-PA, for treating stroke. The

problems include a short therapeutic window and the delayed onset of treatment due to the need for diagnostic testing. For example, as stated in paragraph 9 (emphasis added):

> Early thrombolysis, using intravenous recombinant tissue plasminogen activator (t-PA), is currently the only approved therapy for stroke. The thrombolytic needs to be given within 3.0 hrs of the onset of symptoms and the application of such therapy is severely constrained by the necessity to utilise expensive computerised tomographic (CT) scanning to exclude the possibility of haemorrhagic stroke, for which such agents are contraindicated because they would exacerbate bleeding. With such constraints, it is estimated that around 5% of 500,000 stroke patients currently receive thrombolytic therapy. More recently, two other agents were shown to have therapeutic efficacy: the thrombolytic agent pro-urokinase (r-Pro-UK), delivered by intra-arterial catheter directly to an intravascular thrombus, and the proteolytic enzyme ancrod which has fibrinogen lowering properties. However, both these agents are likely to have the same restricted use as t-PA. (internal citations omitted)

Brearley goes on to provide a solution to the problems associated with single agent therapy: the use of a combination therapy to treat stroke. In paragraphs 482 to 488, Brearley describes additional benefits of the combination therapy. These benefits include: synergistic effects due to the use of two agents, increase in the therapeutic window available for administration of the two agents, and better neuroprotection. However, the use of a combination therapy does not overcome the need for a diagnostic testing prior to administration of a thrombolytic agent. Even as part of a combination therapy, a thrombolytic agent, such as t-PA or pro-UK, cannot be administered to a patient who is suffering a hemorrhagic stroke, because such an agent can exacerbate the bleeding (see, e.g., Brearley paragraph 9).

Further, Brearley provides experimental data demonstrating that in an animal stroke model, therapeutic effects were achieved when a combination therapy, as opposed to single agent therapy, was employed to treat stroke (see, e.g., paragraphs 507, 515, 516). The data demonstrate that the combination therapy extended the therapeutic window for treatment, significantly decreased infarct volume, increased neurological function, and resulted in less body weight loss than single agent therapy. But again, diagnostic testing would be required before a thrombolytic agent could be administered to any patient other than an animal stroke model.

The Office Action at page 5 alleges, "it would have been obvious to one of ordinary skill in the art to use a mutant pro-UK, as disclosed by Liu et al., to treat a stroke patient, because Liu et al. disclose that mutant pro-UK has greatly reduced side effects." Applicants respectfully disagree. First, the systemic bleeding reduction that Liu states his pro-UK mutants provide over native pro-UK does not overcome the problems noted by Brearley. One of skill in the art with knowledge of the pro-UK mutants as described in Liu would not have administered the mutants or the native pro-UK without first confirming an initial diagnosis of stroke using a diagnostic test. It is only the information in the present application that makes the new lifesaving methods of stroke therapy possible.

Furthermore, as stated in the MPEP § 2143.01, "The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination" (emphasis in the original). Even if Brearley could somehow be construed as suggesting the use of a pro-UK mutant from Liu in the treatment of stroke, nothing in Brearley specifically points to the M5 mutant as opposed to one of the other pro-UK mutants described therein. Indeed, even if Brearley could somehow be construed as suggesting the use of a pro-UK mutant, it offers no suggestion that the M5 mutant or any thrombolytic agent could be used without the need for prior diagnostic testing. In contrast, as recited in the claims, M5 can be administered to patients without the need for prior diagnostic testing. Further, although the Liu reference describes its pro-UK mutants as reducing some side effects as compared to pro-UK itself, Liu does not describe or suggest that the M5 mutant could differentiate between good clots and bad clots, or that as a result of this property, M5 can be administered to patients to treat stroke without the need for prior diagnostic testing.

The next reference cited in the Office Action, Pinsky, describes the use of agents (such as CD39 peptides) that inhibit ADP-mediated platelet aggregation by increasing ADP catabolism in the treatment of thrombotic or ischemic disorders, such as stroke. Pinsky describes problems associated with two current thrombolytic agents (t-PA and pro-UK) and the need in the art for an alternative therapy. As stated in paragraph 3 (emphasis added):

> Two thrombolytic agents, recombinant tissue-type plasminogen activator (rtPA) and pro-urokinase, have been used for treatment of stroke. However, their

> therapeutic utility is limited due to risk of symptomatic and fatal intracranial hemorrhage. In the United States, less than 1% of patients presenting to community hospitals with acute ischemic stroke receive rtPA. Inhibition of the final common pathway of platelet accumulation, via blockade of glycoprotein IIb/IIIa receptor-mediated platelet-platelet interactions, does reduce microvascular thrombosis in experimental stroke. However, as with thrombolytic agents, small excesses of a GPIIb/IIIa receptor blocker culminated in serious intracerebral hemorrhage. It is therefore important to identify novel strategies for inhibition of platelet function in acute stroke that will reduce intravascular thrombosis without increasing risk of intracerebral hemorrhage. (internal citations omitted)

It is the present applicants who have discovered this important novel strategy. Pinsky posed the problem, so it may have been obvious to seek a new solution to the problems associated with existing stroke therapies. However, as the Examiner surely knows and as the courts have adjudicated, "obvious to try" is not the standard used for an obviousness determination. For example, in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987) (an invitation to try an experiment is not the proper test for obviousness); *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529 (Fed. Cir. 1988), the Federal Circuit held:

> The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art ... (citations omitted) Both the suggestion and the expectation of success must be found in the prior art, not in the applicant's disclosure.

Applicants submit that Pinsky fails to motivate a skilled practitioner to practice the methods recited in the claims, because Pinsky makes no mention of pro-UK mutants, much less of the M5 mutant, or the potential utility of such mutants in the treatment of stroke. Further, Pinsky actually teaches an alternative solution, the use of CD39 peptides, to the problems that exist with current stroke therapies. Pinksy states that use of CD39 peptides to treat stroke is an effective therapeutic strategy that overcomes problems of existing therapies. For example, as stated in paragraph 104:

> The experimental data indicate that platelet and fibrin deposition in the ipsilateral cerebral hemisphere contribute significantly to the postischemic hypoperfusion and tissue injury which occur in stroke. These studies identify for the first time an in vivo protective role conferred by CD39 in a platelet-dependent thrombotic disorder (stroke). CD39, which we show to be a potent inhibitor of ADP-induced platelet aggregation, also has an extended in vivo half life (elimination half-time in mice is 2 days). Not only does it improve cerebral blood flow and reduce cerebral infarction volumes when given preoperatively, but it also confers significant cerebroprotection when given 3 hours after the onset of stroke The effect of this agent in conferring cerebroprotection at this delayed time point is both novel and important because the cerebroprotective effects occurred without increasing intracerebral hemorrhage or mortality. (internal citations omitted)

Pinksy states that CD39 therapy has benefits over existing stroke treatments, such as a higher therapeutic index, a low risk of causing hemorrhage, and a longer therapeutic window for treatment. As stated in paragraph 109:

> It has been shown that the therapeutic index of CD39 is high, i.e., even twice the effective dose does not increase the occurrence of intracerebral hemorrhage. Furthermore, when given even 3 hours following stroke, therapeutic efficacy is apparent. These data confirm previous research in which microvascular thrombosis was demonstrated to be an ongoing process after the onset of stroke. Inhibition of ongoing microvascular thrombosis is the therapeutic target of the current CD39 strategy. These results are especially important in light of current clinical observation, which show increased intracerebral hemorrhage and mortality if a thrombolytic agent is administered beyond three hours following the onset of stroke. Even in the best of circumstances, few patients arrive at an emergency room in sufficient time to qualify for thrombolytic therapy. An extended time window for administration of a therapeutically useful agent may be an important first step towards improving the current limited treatment paradigms for evolving stroke. (internal citations omitted)

Thus, applicants submit that because Pinsky extols the benefits of using CD39 peptide therapy and recites advantages of this therapy over thrombolytic therapies, this reference fails to provide motivation to use other thrombolytic agents in the presently claimed methods, and certainly does not mention the specific M5 pro-UK mutant, in the treatment of stroke.

The Office Action alleges at page 5:

> The strategy of Pinsky is to identify agents that inhibit platelet aggregation or fibrin deposition in ischemic tissue to treat stroke victims. But, it would have been obvious to one of ordinary skill in the art to pursue other strategies, such as using a form of a drug for treating stroke that has a greatly reduced risk of causing bleeding, such as the mutant pro-UK of Liu.

Applicants respectfully disagree. Although Pinsky sought out agents that inhibit platelet aggregation as a strategy to treat stroke victims, this is irrelevant to the methods recited in the claims, because the methods of the claims use a thrombolytic agent to treat stroke, not a platelet aggregation inhibitor. Because Pinsky identified inhibitors of platelet function, nothing in Pinsky suggests the use of a pro-UK mutant such as M5, or the use of any thrombolytic agent, for the treatment of stroke. Furthermore, nothing in Pinsky specifically points to the M5 mutant described in Liu as opposed to one of the other pro-UK mutants described therein. Pinksy also does not describe any thrombolytic agents that can be administered to a patient without the need for prior diagnostic testing. Pinsky's solution to the problems associated with thrombolytic agents was to use inhibitors of platelet function, which do not dissolve blood clots. Next, as discussed above, Liu fails to describe or suggest that the M5 mutant can differentiate between good clots and bad clots, or that M5 can be administered to patients without the need for prior diagnostic testing. Finally, and contrary to the allegation of the Office Action, because Pinsky provides an alternative method of treatment (i.e., the use of inhibitors of platelet aggregation) that is effective in treating stroke, a skilled practitioner would not have been motivated "to pursue other strategies."

Applicants submit that nothing in Liu, Brearley, or Pinsky, alone or in combination, would have motivated a skilled practitioner to practice the methods recited in the claims. Specifically, none of the references point to use of the M5 mutant in the treatment of stroke, or suggest that the M5 mutant could be administered to patients without prior diagnostic testing. Nothing in the cited references would have suggested the desirability of the combination recited in the claims. Thus, for at least these reasons, applicants respectfully request that the obviousness rejection of claims 1 and 3-6 be withdrawn.

Claim 2

The Office Action alleges that claim 2 is obvious in light of the combination of Liu, Brearley, Pinsky, Barnwell et al. (*Am. J. Rad.* 15:1817-1822 (1994)), and Parsons et al. (*Ann. Neurol.* 51:28-37 (2001)). Applicants respectfully disagree and submit that the Office has failed to establish a *prima facie* case of obviousness with respect to this claim, because the combination of references fails to provide a motivation to combine these references.

The teachings of Liu, Brearley, and Pinsky references have been discussed above and applicants submit that Barnwell and Parsons do not make up for the deficiencies of these three references. None of the cited references, alone or in combination, would have motivated a skilled practitioner to combine the cited references to arrive at the methods recited in the claim.

Parsons describes the use of two imaging techniques to identify which patients may benefit from thrombolytic therapy up to six hours after the onset of a stroke. As stated on page 36, "[w]e therefore suggest that PWI-DWI mismatch [comparison of the two imaging techniques] is a practical way of defining tissue at risk and may enable stroke physicians to rationally select patients for thrombolysis beyond the accepted 3-hour window." This reference is devoid of any motivation to practice the method recited in the claim.

The Office Action at pages 6-7 alleges that:

> It would have been obvious to one of ordinary skill in the art to administer the mutant pro-UK drug of Liu et al. to a patient more than three hours after the onset of stroke symptoms, because Parsons et al. teach that, if the patient is found to have a PWI/DWI imaging mismatch, the patient benefits from the drug because there is partial recovery of the tissue in the area of the stroke lesion. The recovery of penumbral tissue is due to the thrombolytic activity of the drug. Thus, one of ordinary skill in the art would have recognized that a drug with thrombolytic activity may be administered 3-6 hours following the onset of stroke symptoms; the drug need not be t-PA. Another drug with the desired thrombolytic properties that does not cause excessive bleeding, such as the mutant pro-UK of Liu et al. would be expected to work.

However, as discussed above, the mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination (MPEP § 2143.01), and obvious to try is not the proper standard for an obviousness

determination. Because claim 2 depends from claim 1, applicants submit that claim 2 is patentable for the same reasons as discussed above. Applicants submit that nothing in Parsons, alone or in combination with the other cited references, suggests the use of the M5 mutant in the treatment of stroke without first doing a diagnostic test to determine the cause of the stroke.

Barnwell describes the results of a study in which urokinase was administered to patients 3.5 to 48 hours after the onset of a stroke. As stated on pages 1819-1820, "intraarterial urokinase given by selective catheterization during an acute stroke produced a high rate of cerebral vessel recanalization with a relatively low rate of adverse side effects." This reference would not have motivated a skilled practitioner to look for an alternative treatment to current stroke therapies, because it describes the successful use of urokinase to treat stroke, even 3.5 to 48 hours after the onset of a stroke. In addition, this reference describes urokinase therapy as causing minimal side effects. A skilled practitioner reading Barnwell would not have been motivated to seek an alternative treatment.

The Office Action at page 7 alleges that:

> It would have been obvious to one of ordinary skill in the art to administer the mutant pro-UK drug of Liu et al. to a patient more than three hours after the onset of stroke symptoms because Barnwell et al. teach that administration of urokinase more than three hours after the onset of stroke symptoms is effective in most cases if delivered to the area of the lesion. The improvements in the patients are due to the thrombolytic activity of the drug. Thus, one of ordinary skill in the art would have recognized that an improved urokinase, such as the mutant pro-UK of Liu et al., may be administered with efficacy more than three hours following the onset of stroke symptoms. A urokinase with the desired thrombolytic properties ... such as the mutant pro-UK of Liu et al. would be expected to work at least as well as the urokinase of Barnwell et al.

However, because claim 2 depends from claim 1, applicants submit that claim 2 is patentable for the same reasons as discussed above. Applicants submit that nothing in Barnwell, alone or in combination with the other cited references, suggests the use of the M5 mutant in the treatment of stroke.

Applicants submit that nothing in Liu, Brearley, Pinsky, Parsons, or Barnwell, alone or in combination, would have motivated a skilled practitioner to practice the method recited in

claim 2. For at least these reasons, applicants respectfully request that the obviousness rejection of claim 2 be withdrawn.

CONCLUSION

Applicants respectfully submit that in light of the arguments presented herein, the rejections of the pending claims have been overcome, and that the claims are in condition for allowance.

No fees are believed due. Please apply any charges or credits to deposit account 06-1050, referencing Attorney Docket No. 15702-004001.

Respectfully submitted,

Date: 12-27-2005

J. Peter Fasse
Reg. No. 32,983

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

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